Shelf-life evaluation of sliced cold-smoked rainbow trout (*Oncorhynchus mykiss*) under vacuum (PV) and modified atmosphere packaging (MAP).

Lucilla Iacumin[1], Erica Tirloni[2], Marisa Manzano[1], Giuseppe Comi[1*]

[1]Department of Food Science, University of Udine, Via Sondrio 2/a, 33100 Udine, Italy.

[2]Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, via Celoria 10, 20133, Milano, Italy

Number of pages : 21

Number of tables : 3

*Corresponding author: giuseppe.comi@uniud.it - +390432558129 – Fax. +390432558130

Abstract

Cold smoked trout (***Oncorhynchus mykiss***) is a traditional product from the San Daniele del Friuli area (Italy), usually packaged under vacuum, and its shelf life is 60 days. Aim of this study was to evaluate the influence of two different packaging systems (VP and MAP) on the microbial, chemical-physical and sensorial shelf life of sliced cold smoked rainbow trout, outlining the differences between the two methods. MAP packaging was found to better preserve the product, ensuring lower microbial loads throughout storage. VP packaged samples, in particular, exceeded the threshold limit of 6 log CFU/g after 60 days of storage. The TVB-N values, which were quite high at the beginning of the storage, increased over time in both treatments, reaching values close to the limit of 40 mg N/100 g after 45 days. Also TBARS values did not were up to 10 mmol/g in both VP and MAP. Based on the obtained data, the shelf life of 60 days was too long, particularly for the VP samples. A panel composed of 12 non-professional assessors established that a significant difference existed in the sensorial characteristic of the samples, thereby confirming the MAP packaged products had higher sensorial quality than the VP ones.

Keywords: cold smoked Rainbow trout, MAP, vacuum packaging, TVB-N, shelf life

Introduction

Smoked rainbow trout (***Oncorhynchus mykiss***) is a traditional product of Friuli, a region of northeastern Italy. Rainbow trout is farmed in that region, processed by cold-smoking and packaged after slicing under vacuum. The product is stored at 4 ± 2°C and has a shelf life of approximately 60 days. Being a cold-smoked fish, the product belongs to the category of mildly processed foods (Truelstrup Hansen and Huss, 1998). Fish is one of the most perishable food products: its initial microbial population reflects the microflora of the environment at the time of capture or harvest, and it is modified by the ability of various microorganisms (mainly bacteria) to multiply in the sub-environments provided by the skin/shell surface, gill areas, and intestinal content. The muscle tissue is normally sterile: microorganisms can be found on the skin and gills and in the intestinal tract (Baross and Liston, 1970; Shewan, 1977). The microbial loads depend on the water conditions and temperature: during storage, the microflora change, thus allowing the bacteria to tolerate the preservation conditions. The microbial ecology of cold-smoked fishery products has been intensively studied (Bernardi et al. 2009; Gonzàles-Rodrìguez *et al*., 2002; Joffraud *et al.,* 2006; Lannelongue *et al*.,1982; Leroi et al. 1998, 2001; Leroi, 2010; Lyhs *et al*.,1998; Truelstrup Hansen and Huss, 1998). The initial bacterial load closely depends on the hygienic conditions of processing and production (Leroi *et al*., 2001; Truelstrup Hansen and Huss, 1998), and it is represented by lactic acid bacteria (LAB), *Enterobacteriaceae, Shewanella putrefaciens, Aeromonas* spp., *Pseudomonas* spp., *Photobacterium phosphoreum*, and *Brochothrix thermosphacta* (Cardinal *et al*., 2004; Civera *et al*., 1995; Jorgensen and Huss, 1989; Leroi *et al.,* 2001, 2010; Lyhs *et al*., 2007; Jaffrès *et al*., 2008; Laurse, et al., 2005; Truelstrup Hansen *et al*, 1996).

The refrigeration of cold smoked salmon and trout does not prevent the growth of abundant microflora, particularly LAB, which have a focal role in the microbial evolution occurring in the product. The shelf life of several cold smoked fish products is limited by the presence and growth of specific microflora, and although a certain correlation between these microflora and spoilage development has not yet been established, a level of microorganisms approximately 7-8 log CFU/g is considered to determine consumer rejection. These microflora could also be present at high loads without causing product spoilage; other factors are responsible for important microbial spoilage (Gram,1991; Gonzàles-Rodrìguez *et al* , 2002; Joffraud *et al* , 2001).

The dominant microflora in both vacuum-packed and MAP-packed smoked fish at the end of the retention period was often found to be LAB: the predominance of this group is not totally clear, but it seems that they are well suited to the particular pH, water phase salt (WPS) and salt concentration of smoked products (Bernardi *et al*., 2009; Cardinal *et al*., 2004; Laursen *et al*., 2005; Leroi *et al*., 2000; Lyhs *et al*., 2007; Samelis *et al*., 1994). Also *Pseudomonas* spp. could be cause of spoilage in VP due to residual oxygen or to oxygen permeability of the protective film. *Pseudomonas* spp. could be the result of the initial contamination of the raw material. *Enterobacteriaceae* (Bernardi *et al*., 2009; Gimenez and Dalgaard, 2004; Leroi, 2010) could be also present, they can growth either in VP or in MAP because they are anaerobic facultative, and consequently can produce acids and gas (CO_2). Among them *Enterobacter* spp., *Serratia* spp., *Hafnia* spp. and *Proteus* spp. are the bacteria mainly present and are indicative of the level of hygiene in the processing (Bernardi *et al*., 2009). In the case of smoked salmon and related products, such as smoked trout, their role has not yet been clarified and they seem to have a minor role in the process of spoilage; many authors believe that there is no direct correlation between shelf-life and total counts of LAB (Leroi *et al*.,

2001), even though the LAB cause spoilage with the production of volatile sulphur compounds and amines (Bernardi *et al.*, 2011). The LAB, however, should not be considered harmful exclusively for the purpose of preservation, as they represent an important ally against potentially pathogenic microorganisms. Many studies (Gimenez and Dalgard, 2004; Joffraud *et al.*, 2006; Leroi, 2010; Leroi *et al.*, 1996) have reported that the presence of LAB, or their inoculation in smoked fishery products, results in growth inhibition of *Listeria monocytogenes*, *Clostridium botulinum* type E and *Salmonella* spp. This paper focuses on cold smoked trout fillets, a traditional product of the San Daniele del Friuli area that is usually packaged under vacuum (UV) after slicing.

For the convenience of the consumer, because modified atmosphere packaging (MAP) allows for the easier separation of the slices, this study compared the shelf life of sliced cold-smoked rainbow trout packaged under vacuum to that of the same product with MAP packaging.

Material and Methods

Samples

Samples consisted of portions (200 g) of sliced cold-smoked rainbow trout packaged under vacuum (- 1.0 bar) or in MAP (- 0.3 bar; 70% N_2 and 30% CO_2) , by Orved VM53 vacuum machine (Italy). The packaging consisted of Ecoterm VP 300 film (vacuum) and Multofog GA 170 (MAP; SUDPACK, Italy). The packages were stored at 4 ± 2 °C for 60 days and analysed after 0, 15, 30, 45, and 60 days. Three packages at each time were used for microbiological and physico-chemical analyses. To produce the sliced cold-smoked raimbow trout used in the tests., two lots of raw material, were used. The trouts were farmed by Italian farm of San Daniele area (Friuli, Italy), they sized of 55 ± 5 cm , weighted 4.5 ± 5 kg, were put in ice water bath and beheaded, the post-mortem

period was about 24 h at 4 °C. Then they were smoked in smoking rooms with smoke temperature about 29 °C for 24 h.

Microbiological analysis

Total aerobic mesophilic microbial counts (TMC) were evaluated on plate count agar (Oxoid, Italy) incubated at 30 °C for 48-72 h. LAB were counted onto De Man Rogosa Sharpe (MRS, Oxoid, Italy) after incubation under anaerobic conditions at 30 °C for 48 h; yeasts and moulds were counted onto malt agar (MA) (Oxoid, Italy) after incubation at 25 °C for 72-96 h. Total coliforms were counted on violet red bile lactose agar (VRBLA, Oxoid, Italy) and incubated at 37 °C for 24 h; coagulase-positive Staphylococci were enumerated on Baird-Parker agar medium (BP, Oxoid, Italy) supplemented with egg yolk tellurite emulsion (Oxoid, Italy) and incubated at 35 °C for 24-48 h; *Pseudomonas* spp. were counted on Pseudomonas agar base (Oxoid, Italy) supplemented with CFC (Oxoid, Italy) and incubated at 30 °C for 48 h; sulphite-reducing Clostridia were quantified on differential reinforced clostridial medium (DRCM, VWR, USA) after incubation at 37 °C for 24-48 h in an anaerobic jar using an anaerobic kit (gas pack anaerobic system, BBL, Becton Dickinson, USA). *Listeria monocytogenes* and *Salmonella* spp. were investigated according to ISO methods 11290/1 and 6579-1, respectively.

Physico-chemical analysis

The pH value was measured using a pH meter (Basic 20, Crison Instruments, Spain), inserting the pH meter probe into 3 different points on each sample. Aw (water activity) was measured by Aqua Lab 4 TE (Decagon Devices, USA). The moisture content was measured by the A.O.A.C. (1990) method, NaCl and TVB-N (total volatile basic nitrogen) content were measured by the Pearson

122 method (1973). WPS was determined by the formula WPS = % salt/(% salt + % moisture) x 100, as
123 described by Huss *et al.*, (1997). Analyses were performed in triplicate per each sampling point. To
124 evaluate the oxidation stability during storage, the Thiobarbituric acid–reactive substances
125 (TBARS) were used in triplicate (Ke et al., 1984).
126
127 *Sensorial analysis*
128 To evaluate the influence of UV and MAP packaging on the organoleptic characteristics of the
129 sliced cold-smoked rainbow trout samples, a sensory analysis was performed using the triangle test
130 methodology (ISO 4120:2004). Sensorial analyses were performed by 12 non-professional
131 assessors. Four additional samples per each treatment were also evaluated after 60 days of storage
132 at 4 ± 2 °C. The non-professional assessors were presented with three products, two of which were
133 identical. The assessors were asked to state which product they believed was the odd one out. The
134 assessors who identified the different samples were asked to indicate their preference.
135
136 *Statistical analysis*
137 The values of the various parameters were compared by one-way analysis of variance. The averages
138 were compared with Tukey's honest significance test using the Statistical Graphics software
139 package.
140
141 **Results and Discussion**
142 The loads obtained from microbiological analyses of cold smoked trout fillets packaged under VP
143 or in MAP are reported in Table 1. In the VP samples, the TMC was below the detection limit

144 (<100 CFU/g) at day 0, but a rapid increase after T15 was found, reaching at the end of the storage
145 period a microbial count higher than the threshold limit of 6 log CFU/g often used in food industries
146 to indicate the end of shelf life of fish products (Olafsdottir *et al*., 2005). Conversely, in samples
147 under MAP, the TMC was slightly higher at day 0, but the loads remained almost stable
148 (approximately 2-3 log CFU/g) until day 45, at which point a rapid increase to a value of 4.52±0.43
149 log CFU/g at day 60 was observed. It needs to be highlighted that a TMC of 7-8 log CFU/g, often
150 associated with sensory rejection, was never reached. From T15 until the end of the storage period,
151 the TMC values were significantly higher for the VP samples than for the MAP samples ($p < 0.05$).
152 Exactly the same trend was observed for the LAB counts. In fact, a rapid increase was observed
153 during the storage period, with a jump of approximately 3 log from day 0 to day 15 and another
154 jump of approximately 3 log from day 45 to day 60, when they reached a concentration of 6.42 ±
155 0.67 log CFU/g. Further, in this case, the LAB counts were significantly different between the
156 treatments, thus confirming significantly higher values in the VP samples compared with the MAP
157 samples ($p< 0.05$), which were characterised by limited LAB growth throughout the period. Our
158 values are similar or slightly lower than those reported in the literature for smoked vacuum-packed
159 salmon: in those studies, the TMC values were approximately 7 to 8 log CFU/g, and LAB levels
160 were between 5 and 6 log CFU/g (Bernardi *et al.*, 2009; Jaffrès *et al.*, 2008; Leroi *et al.*, 1998,
161 2001; Leroi, 2010; Truelstrup Hansen *et al*., 1996; Truelstrup Hansen and Huss, 1998). Dondero *et*
162 *al*., (2004) and Gonzàles-Rodrìguez *et al.*, (2002) obtained similar results in cold-smoked salmon
163 and trout, even if the initial bacterial load, an index of the quality of the raw material and the
164 hygiene of the process, was lower in our work than in these studies.

High-quality raw materials and good manufacturing practices were confirmed by the values of Coliforms and coagulase-positive Staphylococci (1 log CFU/g), which were always below the detection limit in both the VP and MAP samples during the monitored period, and by the absence of *Salmonella* spp. and *Listeria monocytogenes* in all samples.

The different types of packaging had an effect on *Pseudomona*s spp. growth (Table 1), particularly at the last two time points, where a rapid increase in the VP samples was observed. Conversely, in the MAP samples, *Pseudomonas* spp. were counted only at day 15 (2.00 ± 0.07), then they were no longer detected until the end of the storage period (< 100 CFU/g). The contamination could either derive from the processing or originate from the raw materials.

According to data obtained from previous studies, their growth depends on the oxygen permeability of the vacuum packaging (Gonzàles-Rodrìguez *et al*., 2002; Leroi *et al*., 1998, 2001). Sulphite-reducing clostridia were found only at day 60 in the VP samples. This result suggests that their presence cannot be connected to real growth but was likely sample-dependent.

Considering the physico-chemical analyses (Table 2), both pH and Aw showed a slight decrease over time without significant differences between the VP and MAP samples. The pH values started from 6.16 in both the VP and MAP products with no significant differences ($p > 0.05$) during the storage period. Only at the end of the shelf life (T60), the pH decreased to 6.03 and 5.97 in the VP and MAP samples, respectively, and a significant difference was noted ($p < 0.05$); this slight decrease could be due to the increase of Lactic Acid Bacteria that generally exert a buffer activity onto the food substrate. Leroi et al.. (1998) found quite stable pH values (from 6.04 to 6.25), as did Bernardi *et al.*, (2009), who reported constant values in the middle and at the end of the shelf life (6.12-6.11) in their study.

187 Similarly, the Aw values detected in the MAP samples were slightly lower than those measured for
188 the VP samples, but the noticed differences were only significant at days 15 and 60 ($p < 0.05$). The
189 small observed Aw differences at the various sampling points probably depend on the samples'
190 variability and are not related to real water loss. Moisture remained fairly constant over time. As far
191 as the salt content and the WPS are concerned, the literature emphasises the importance of
192 considering a WPS value of 3.5% as the minimum value able to control *Clostridium botulinum*, and
193 in particular psychotropic type E, when combined with storage temperatures below 4.4 °C (Centre
194 for Food Safety and Applied Nutrition, 2001). In this study, the values of salt and WPS varied
195 during the storage period without displaying a specific trend, indicating that the observed
196 differences depend only on the variability of the samples. The salt content was affected by the
197 variability of the samples and of the salting procedure; for these reasons the decrease observed
198 could not be considered a trend but a random heterogeneity of the samples.
199 Most of the studies met the guidelines suggested by the Centre for Food Safety and Applied
200 Nutrition: Bernardi *et al.*, (2009) reported a mean WPS value in Italian smoked salmon products
201 equal to 4.93%, whereas in French products the averages were lower but still approximately 4%
202 (Cornu *et al.*, 2006; Hespe *et al.*, 2004). Moreover, differently from what found in the present study,
203 Bernardi *et al.*, (2009) highlighted that WPS values lower than 3.5% were correlated with low
204 bacterial counts. The mean TBARS values did not increased up to 10 nmol/g at the end of the
205 storage (60 days) either in PV or in MAP samples. According to different authors (Ke *et al.*, 1984;
206 Che Man and Ramadas, 1998) for quality evaluation, TBARS values of < 8 nmol/g sample are
207 considered not rancid, 9– 20 nmol/g slightly rancid but still acceptable and >21 nmol/g rancid and
208 unacceptable, consequently for the TBARS values, both the VP and MAP samples tested can be

209 considered still acceptable at 60 days, despite at 60 days 10 out of 12 assessors perceived a light
210 rancidity and fluid loss in PV products and only 1 out of 12 perceived a light rancidity in MAP
211 products. In PV products, the TBARS means at 60 days were significantly different compared to the
212 ones at 0,15,30,45 days. In contrast no significant difference was present among TBARS means in
213 MAP products at each tested times.
214 Considering the TVB-N results (Table 2), a moderate increase over time in both the treatments was
215 observed. As early as day 45, the values were close to 40 mg N/100 g, which corresponds to the
216 maximum value proposed by Cantoni *et al.,* (1993) for this parameter, with no significant
217 differences observed between the treatments. Additionally, Bernardi *et al*., (2009) found that at half
218 the shelf life, the TVB-N values were close to the initial TVB-N limit (38.2 mg N/100 g), and at the
219 end, they largely exceeded the suggested maximum value (49.8 mg N/100 g). The Chilean
220 authorities (Sernapesca, 1996) established a limit of 30 mg N/100 g for cold smoked salmon, and
221 this value was just present in our trial at day 0 for both the VP and MAP products. The observed
222 TVB-N data were different from those reported by Leroi *et al.,* (1998), who found initial TVB-N
223 values of approximately 15.5 mg N/100 g and final higher values equal to 52.8 mg N/100 g.
224 A multiple compound quality index was proposed by Leroi *et al.,* (2001) to estimate the remaining
225 shelf life time considering TVB-N and *Lactobacillus* spp. loads at storage temperatures < 5 °C.
226 Applying this model and using the data of T30 for the VP and MAP products, the remaining shelf
227 life was found to be 52 days and 55 days, respectively.
228 Considering the threshold limit of 6 log CFU/g (Olafsdottir *et al.,* 2005) for TMC and the TVB-N
229 limit of 40 mg N/100 g (Cantoni *et al.,* 1993), the MAP products can be accepted until the end of
230 their shelf life (60 days), but because the VP products exceeded the threshold limit of 6 log CFU/g,

231 this shelf life period is not adequate. MAP allowed to maintain higher structural and physical
232 qualities than VP, wherein higher fluid loss occurred and less juiciness was noticed. However, the
233 MAP composition must be carefully studied, as it can create unfavourable conditions for the
234 microflora, hindering their ability to compete with pathogens, the replication of which may be
235 facilitated to in this substrate, e.g., *C. botulinum* under anaerobic conditions (Huss, 1980),
236 especially without a proper salt concentration.
237 The sensorial acceptability of both types of smoked rainbow trout, packaged VP or in MAP, was
238 determined by the triangular test. The panel, which was composed of 12 non-professional assessors,
239 established that a difference exists in the sensorial characteristic between the trout packaged in VP
240 versus that in MAP, thereby confirming the physico-chemical and microbiological results. All the
241 assessors indicated there were two distinct samples and identified that the MAP products had a
242 higher sensorial quality than the VP (Table 3) also at the end of shelf-life.
243
244 **Conclusion**
245 The sliced smoked rainbow trout samples, both vacuum and MAP packaged, were found to have
246 good hygienic quality and to comply with the EC Regulation no. 2073/2005 as *Listeria*
247 *monocytogenes*, *Salmonella* spp., coagulase-positive Staphylococci and Coliforms were never
248 detected. MAP packaging preserved the product better compared with vacuum packaging, showing
249 lower microbial loads throughout the storage period and at the end of shelf life.
250 However, some samples showed less than 3.5% WPS, the limit for the growth of psychrotrophic
251 (non-proteolytic) *Clostridium botulinum*. As this bacterium could grow during the product's 60-day

252 commercial life, it is essential to optimise salting during the production process to ensure that an
253 adequate concentration of NaCl is distributed homogenously throughout the raw material.
254 The deadline of 60 days appears to be too long, particularly for the VP samples, as confirmed by the
255 sensory analysis. Consequently, a reduction of the "use-by" date could allow the manufacturer to
256 guarantee the quality and safety of this product for its entire commercial life. For PV products a
257 shelf-life of 45 days is more reliable.
258
259 **References**

260 A.O.A.C. 1990. Official Methods of analysis of the A.O.A.C., 15th edn. A.O.A.C., Arlington, USA.
261 Baross, J., and Liston, J. 1970. Occurrence of *Vibrio parahaemolyticus* and related haemolytic
262 vibrios in marine environments of Washington State. Applied Microbiology, 20: 179-186.
263 Bernardi, C., Ripamonti, B., Campagnoli, A., Stella, S., and Cattaneo, P. 2009. Shelf-life of vacuum
264 packed Alaskan, Scottish and Norwegian cold-smoked salmon available on the Italian market.
265 International Journal Food Science and Technology, 44**:** 2538-2546. doi:10.1111/j.1365-
266 2621.2009.02081.x.
267 Bernardi, C., Ripamonti, B., Marzano, M.A., and Cattaneo, P. 2011. Aspetti critici nella produzione
268 di ritagli di salmone affumicato. A.I.V.I online Giugno, 1 (0): 145-148.
269 Cantoni, C., Moret, S., and Comi, G. 1993. Bacteriological and chemical indices to evaluate
270 smoked salmon quality. Industria Alimentare XXXII (September), 842-845.
271 Cardinal, M., Gunnlaugsdottir, H., Bjoernevik, M., Ouisse, A., Vallet, J. L., and Leroi, F. 2004.
272 Sensory characteristics of cold-smoked Atlantic Salmon (*Salmo salar*) from European market
273 and relationships with chemical, physical, and microbiological measurements. Food Research

International, 37: 181-193. doi:10.1016/j.foodres.2003.12.006.

Centre for Food Safety and Applied Nutrition 2001. Processing parameters needed to control pathogens in cold smoked fish. U.S. Food and Drug Administration. http:/www.fda.gov/Food/ScienceResearch/resaarchArea/SafePracticesforFoodProcesses/ucm092182.htm.

Che Man, Y.B. & Ramadas, J. (1998). Effect of packaging environ- ment on quality changes of smoked Spanish mackerel under refrigeration. Journal of Food Quality, 21, 167–174. doi: 10.1111/j.1745-4557.1998.tb00513.x

Civera, B., Parisi, E., Amerio, G.P., and Giaccone, V. 1995. Shelf-life of vacuum packed smoked salmon: microbiological and chemical changes during storage. Archives fur Lebensmithygiene, 46: 1-24.

Cornu, M., Beaufort, A., Rudelle, S., Laloux, L., Bergis, H., Miconnet, N., Serot, T., and Delignette Muller, M.L. 2006. Effect of temperature, water-phase salt and phenolic contents on *Listeria monocytogenes* growth rates on cold-smoked salmon and evaluation of secondary models. Int. Journal of Food Microbiology, 106, 159-168. doi:10.1016/j.ijfoodmicro.2005.06.017.

Dondero, M., Cisternas, F., Carvajal, L., Simpson, R. 2004. Change in quality of vacuum-packed cold-smoked salmon (*Salmo salar*) as a function of storage temperature. Food Chemistry, 87**:** 543-550. 0. doi:10.1016/j.foodchem.2004.01.005

Gimenéz, B., and Dalgaard, P. 2004. Modelling and predicting the sumultaneous growth of *Listeria monocytogenes* and spoilage micro-organism in cold-smoked salmon. Journal of Applied Microbiology, 96: 96-109. doi: 10.1046/j.1365-2672.2003.02137.x

295 Gonzàlez- Rodríguez, M.N., Sanz, J.J., Santos, J.Á., Otero, A., and García-López, M.L. 2002.
296 Numbers and types of microorganisms in vacuum-packed cold-smoked freshwater fish at the
297 retail level. International Journal of Food Microbiology, 77: 161-168.
298 Gram, L. 1991. Inhibition of mesophilic spoilage *Aeromonas* spp. On fish by salt, potassium
299 sorbate, liquide smoke, and chilling. Journal of Food Protection, 54: 436-442.
300 Hespe, M., Kiessling, A., Lunestad, B.T., Torrissen, O.J., and Bencze Røra, A.M. 2004. Quality of
301 cold-smoked collected in one French Hypermarket during a periode of 1 year. Lebensmittel
302 Wissenschaft Technologie, 37: 617-638. 638.doi:10.1016/j.lwt.2004.01.008
303 Huss, H.H. 1980. Distribution of *Clostridium botulinum*. Applied Environmental Microbiology, 39:
304 764-769.
305 Huss, H.H., Dalgaard, P., and Gram, L. 1997. Microbiology of fish and fish products. *In* Seafood
306 from Producer to Consumer, Integrated Approach to Quality. *Edited by* J. B. Luten,T.
307 Borresen and J. Oehlenschläger. Amsterdam, (NL): Elsevier Science B.V. pp. 413 – 430.
308 ISO 11290-1:1996 Adm.1:2004. Microbiology of food and animal feeding stuffs – Horizontal
309 method for the detection of *Listeria monocytogenes*.
310 ISO 6579-1: 2002 Cor.1:2004 Microbiology of food and animal feeding stuffs – Horizontal method
311 for the detection of *Salmonella* spp.
312 ISO 4120:2004. Triangle test methodology. Standard test method for sensory analysis — General
313 guidance for the design of test rooms.
314 Jaffrès, E., Sohier, D., Leroi, F., Pilet, M. F., Prévost, H., Joffraud J.J., and Dousset, X. 2008. Study
315 of the bacterial ecosystem in tropical cooked and peeled shrimps using a polyphasic approach.

316 International Journal of Food Microbiology, 131: 20-29. doi:10.1016/j.ij food m
317 icro.2008.05.017
318 Joffraud, J.J., Leroi, F., Roy, C., and Berdagué, J.L. 2001. Characterisation of volatile compounds
319 produced by bacteria isolated from spoilage flora of cold-smoked salmon International
320 Journal of Food Microbiology, 66: 175-184. 184. doi:10.1016/S0168-1605(00)00532-8
321 Joffraud, J.J., Cardinal, M., Cornet, J., Chasles, J.S., Léon, S., Gigout, F., and Leroi, F. 2006. Effect
322 of bacterial interactions on the spoilage of cold-smoked salmon. International Journal of Food
323 Microbiology, 112: 51-61. -doi:10.1016/j.ijfoodmicro.2006.05.014
324 Jorgensen, B.R.,and Huss, H.H. 1989. Growth and activity of *Shevanella putrefaciens* isolated from
325 spoiling fish. International Journal of Food Microbiology, 9: 51-62. 2. doi:10.1016/0168-
326 1605(89)90037-8
327 Ke, P.Y., Cervantes, E., and Robles-Martınez, C. 1984. Determination of thiobarbituric acid
328 reactive substances (TBARS) in fish tissue by an improved distillation spectrophotometer
329 method. Journal of the Science of Food and Agriculture, 35: 1248–1254. doi:
330 10.1002/jsfa.2740351117
331 Lannelongue, M., Hanna, M.O., Finne, G., Nickelson, R., and Vanderzant, C. 1982. Storage
332 characteristics of finfish fillets (*Archosagus probatocephalus*) packaged in modified gas
333 atmospheres containing carbon dioxide. Journal of Food Protection, 45: 440-444.
334 Laursen, B.G., Bay, L., Cleenwerck, L., Vancanneyt, M., Swings, J., Dalgaard, P., and Leisner, J.J.
335 2005. *Carnobacterium divergens* and *Carnobacterium maltaromicum* as spoilers or protective
336 cultures in meat and seafood: phenotypic and genotypic characterisation. Systematic Applied
337 Microbiology, 28: 151-164. doi:10.1016/j.syapm.2004.12.001

338 Leroi, F., Arbey, N., Joffraud, J.J., and Chevalier, F. 1996. Effect of inoculation with lactic acid
339 bacteria on extending the shelf life of vacuum-packed cold –smocked salmon. International
340 Journal of Food Science and Technology, 31: 497-504. doi:10.1046/j.1365-
341 2621.1996.00366.x
342 Leroi, F., Joffraud, J.J., Chevalier, F., and Cardinal, M. 1998. Study of the microbial ecology of
343 cold-smoked salmon during storage at 8 °C. International Journal of Food Microbiology, 39:
344 111-121. doi:10.1016/S0168-1605(97)00126-8
345 Leroi, F., and Joffraud, J.J. 2000. Salt and smoke simultaneously affect chemical and sensory
346 quality of cold-smoked salmon during 5 °C storage predicted using factorial design. Journal
347 of Food Protection, 63: 1222-1227.
348 Leroi, F., Joffraud, J.J., Chevalier, F., and Cardinal, M. 2001. Research of quality indices for cold-
349 smoked salmon using a stepwise multiple regression of microbiological count and physico-
350 chemical parameters. Journal of Applied Microbiology, 90: 578-587. doi: 10.1046/j.1365-
351 2672.2001.01283.x
352 Leroi, F. 2010. Occurrence and role of lactic acid bacteria in seafood products. Food Microbiology,
353 27: 698-709. doi:10.1016/j.fm.2010.05.016.
354 Lyhs, U., Björkroth, J., Hyytiä, E., and Korkeala, H. 1998. The spoilage flora of vacuum-packaged,
355 sodium nitrite or potassium nitrate treated, cold-smoked rainbow trout stored at 4 °C or 8 °C.
356 International Journal of Food Microbiology, 45: 135-142. doi:10.1016/S0168-
357 1605(98)00160-3

358 Lyhs, U., Lahtinen, J., and Schelvis Smith, R. 2007. Microbiological quality of maatjes herring
359 stored in air and under modified atmosphere at 4 and 10 degrees C. Food Microbiology, 24:
360 508-516. doi:10.1016/j.fm.2006.08.003
361 Olafsdottir, G., Chaine, E., Westad, F., et al. 2005. Prediction of microbial and sensory quality of
362 cold smoked Atlantic salmon (*Salmo salar*) by Electronic Nose. Journal of Food Science, 70:
363 563-574. doi: 10.1111/j.1365-2621.2005.tb08330.x
364 Pearson, D. 1973. Laboratory Techniques in Food Analysis. Butterworths & Co. Publishers Ltd.,
365 London, UK, pp. 169 and 201-202.
366 Samelis, J., Maurogenakis, F., and Metaxopoulos, J. 1994. Characterization of lactic acid bacteria
367 isolated from naturally fermented Greek dry salami. International Journal of Food
368 Microbiology, 23: 179-196. doi:10.1016/0168-1605(94)90051-5
369 Sernapesca 1996. Programa de certificación de producto final, Norma técnica. CER/NT/95.
370 Shewan, J.M. 1977. The bacteriology of fresh and spoiling fish and the biochemical changes
371 induced by bacterial action. In Proceeding of the Conference on Handling, Processing and
372 Marketing of Tropical Fish. London: Tropical Products Institute, pp. 51-66.
373 Truelstrup Hansen, L., Gill, T., Drewes Røntved, S., and Huss, H.H. 1996. Importance of autolysis
374 and microbiological activity on quality of cold-smoked salmon. Food Research International,
375 29: 181-188. doi:10.1016/0963-9969(96)00003-8
376 Truelstrup Hansen, L., and Huss, H.H. 1998. Comparison of the microflora isolated from spoiled
377 cold-smoked salmon from three smokehouses. Food Research International, 31**:** 703-711.
378 doi:10.1016/S0963-9969(99)00049-6
379

Table 1: Microbiological analysis results (Log CFU/g) of the sliced cold smoked rainbow trout packaged under vacuum (VP) and in Modified Atmosphere (MAP).

Days	Total Mesophilic Count		*Pseudomonas* spp.		Lactic Acid Bacteria		Sulphite-reducing clostridia	
	VP	MAP	VP	MAP	VP	MAP	VP	MAP
0	< 100*	2.70±0.51	< 100*	< 100*	< 10*	1.30±0.41	< 10*	< 10*
15	4.29±0.50$_a$	2.24±0.34$_b$	< 100*	2.00±0.07	3.57±0.95$_a$	1.30±0.27$_b$	< 10*	< 10*
30	4.14±0.42$_a$	2.93±1.31$_b$	< 100*	< 100*	4.14±0.65$_a$	3.37±0.52$_a$	< 10*	< 10*
45	4.78±0.22$_a$	2.50±0.71$_b$	4.34±0.26	< 100*	3.53±1.27$_a$	1.97±1.68$_a$	< 10*	< 10*
60	6.21±0.29$_a$	4.52±0.43$_b$	6.66±0.01	< 100*	6.42±0.67$_a$	4.16±0.61$_b$	2.23±0.31	< 10*

Data represent the means ± standard deviations of the total samples; Mean with the same letters within a row (following the values), considering each single parameter, are not significantly differently ($P < 0.05$).*Value are expressed as CFU/g. Analyses were conducted in triplicate on three different samples per each sampling point.

Table 2: Results of physical-chemical analyses of the sliced, cold smoked rainbow trout packaged under vacuum (VP) and under Modified Atmosphere (MAP).

		Days				
		0	**15**	**30**	**45**	**60**
% Water	**VP**	59.84±0.25$_b$	63.64±0.86$_a$	63.31±0.86$_a$	62.43±1.89$_a$	63.59±0.81$_a$
	MAP	61.22±0.10$_a$	63.37±3.04$_a$	61.77±4.27$_a$	64.01±2.10$_a$	62.93±0.61$_a$
% Salt	**VP**	3.11±0.03$_b$	2.18±0.59$_a$	2.69±0.14$_a$	2.78±0.16$_a$	2.59±0.23$_a$
	MAP	3.43±0.12$_a$	2.79±0.23$_a$	2.16±0.05$_b$	2.46±0.29$_a$	2.79±0.28$_a$
pH	**VP**	6.16±0.03$_a$	6.03±0.09$_a$	6.06±0.07$_a$	5.91±0.01$_a$	6.03±0.04$_a$
	MAP	6.16±0.04$_a$	5.98±0.03$_a$	6.16±0.04$_a$	5.96±0.06$_a$	5.97±0.01$_b$
Aw	**VP**	0.973±0.007$_a$	0.971±0.006$_a$	0.966±0.006$_a$	0.967±0.009$_a$	0.966±0.004$_a$
	MAP	0.963±0.008$_a$	0.963±0.001$_b$	0.963±0.004$_a$	0.968±0.002$_a$	0.958±0.002$_b$
% WPS	**VP**	4.94±0.15$_b$	3.32±0.91$_a$	4.07±0.16$_a$	4.27±0.21 $_a$	3.85±0.28$_a$
	MAP	5.31±0.01$_a$	4.23±0.23$_a$	3.39±0.19$_b$	3.72±0.52$_a$	4.24±0.62$_a$
TVB-N	**VP**	32.9±0.33$_a$	37.55±3.55$_a$	35.86±0.87$_a$	40.52±1.28$_a$	39.00±1.19$_a$
mg N/100 g	**MAP**	32.61±1.39$_a$	34.95±1.38$_a$	36.19±0.23$_a$	39.20±1.45$_a$	39.35±0.64$_a$
TBARS	**VP**	7.8 ± 1.2$_a$	8.1 ± 1.2$_a$	8.6 ± 0.5$_a$	9.2 ±0.6$_a$	10.6 ± 03$_b$
nmol/g	**MAP**	7.7 ± 1.3$_a$	7.9.± 0.9$_a$	8.4.± 1.8$_a$	9.3 ± 0.8$_a$	9.5 ± 0.6$_a$

Legend: WPS: Water Salt Phase; TVB-N: Total volatile basic nitrogen; Data represent the means ± standard deviations of the total samples; Mean with the same letters within a column (following the values), considering each single parameter, are not significantly differently (P< 0.05). Analyses were conducted in triplicate on three different samples per each sampling point.

Table 3: Results of the sensorial analysis to determine

the limit of acceptability by 12 assessors

Day	PV		MAP	
	A	NA	A	NA
0	12		12	
15	12		12	
30	12		12	
45	12		12	
60	2	10*	11	1*

Legend: 12 assessors; A: accettable: NA: Not accettable

PV: Vacuum packaged; MAP: Modified Atmosphere

Packaging; 10*: light rancidity/fluid loss/ less juiciness;

1*: light rancidity.